U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-299 464

'Crassostrea virginica' as an Indicator of Lead Pollution

(U.S.) Environmental Research Lab, Narragansett, RI

16 Feb 79

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/J-79-028	2. JOURNAL ARTICLE
3. RE PB 299464	
4. TITLE AND SUBTITLE Crassostrea virginica as an Indicator of Lead Pollution	5. REPORT DATE 1979(Issue Date)
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Zaroogian, G.E., G. Morrison and J.F. Heltshe	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S. Environmental Protection Agency Environmental Research Lab. Narragansett, RI 02882	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS same as above	13. TYPE OF REPORT AND PERIOD COVERED journal article
	14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES
REFERENCE: MARINE BIOLOGY, VOL. 52(2), pp. 189-196, 1979

16. ABSTRACT

After treatment with 1.0 and 3.3 µg Pb kg^{-1} for 20 weeks in flowing seawater at ambient salinity and temperature, *Crassostrea virginica* accumulated as much as 6.57 and 11.42 µg g^{-1} dry weight, respectively, and no mortalities were recorded. Lead uptake was curvilinear; however, the general trend was an increase in lead concentration over time. Dry weight of the oyster had no significant relation with tissue lead concentration (µg g^{-1}); however, a significant positive relationship existed between weight and lead content (µg). A decrease in lead concentration in the tissues occurred from mid-August to mid-September despite continuous addition of lead to the seawater. Addition of lead to the seawater was terminated after 20 weeks (October), and lead loss was studied for the following 12 weeks. Lead loss was studied well after spawning in an attempt to eliminate any influence this might have on lead concentration in the tissues. After 4 weeks, approximately 54% of the accumulated lead was lost under conditions of a natural temperature decline. The biological half-life of lead in *C. virginica* tissues was calculated to be 5.5 weeks. In the ensuing 8 weeks, little or no lead loss was observed. Larvae from each treatment developed to the straight-hinged stage within 48 h with no apparent abnormalities. Lead appeared to have no adverse effect on larvae when parents had been treated with 1.0 and 3.3 µg kg^{-1} seawater for 10 weeks.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Environmental Biology, Water Pollution and Control		

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT Release Unlimited	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES 10
	20. SECURITY CLASS *(This page)*	22. PRICE A02-A01

EPA Form 2220-1 (9-73)

Marine Biology 52, 189-196 (1979)

MARINE BIOLOGY
by Springer-Verlag 1979

EPA-600/J-79-028
JOURNAL ARTICLE

Crassostrea virginica as an Indicator of Lead Pollution

G.E. Zaroogian, G. Morrison and J.F. Heltshe

Environmental Research Laboratory, U.S. Environmental Protection Agency; Narragansett, Rhode Island, USA

Abstract

After treatment with 1.0 and 3.3 µg Pb kg^{-1} for 20 weeks in flowing seawater at ambient salinity and temperature, *Crassostrea virginica* accumulated as much as 6.57 and 11.42 µg g^{-1} dry weight, respectively, and no mortalities were recorded. Lead uptake was curvilinear; however, the general trend was an increase in lead concentration over time. Dry weight of the oyster had no significant relation with tissue lead concentration (µg g^{-1}); however, a significant positive relationship existed between weight and lead content (µg). A decrease in lead concentration in the tissues occurred from mid-August to mid-September despite continuous addition of lead to the seawater. Addition of lead to the seawater was terminated after 20 weeks (October), and lead loss was studied for the following 12 weeks. Lead loss was studied well after spawning in an attempt to eliminate any influence this might have on lead concentration in the tissues. After 4 weeks, approximately 54% of the accumulated lead was lost under conditions of a natural temperature decline. The biological half-life of lead in *C. virginica* tissues was calculated to be 5.5 weeks. In the ensuing 8 weeks, little or no lead loss was observed. Larvae from each treatment developed to the straight-hinged stage within 48 h with no apparent abnormalities. Lead appeared to have no adverse effect on larvae when parents had been treated with 1.0 and 3.3 µg kg^{-1} seawater for 10 weeks.

Introduction

Contamination of the marine environment with lead is a growing problem. Lead "antiknock" compounds used in automobile fuels are the principle anthropogenic sources of lead in the environment. Lead enters the atmosphere via automobile exhausts and later precipitates out onto land and water. A tremendous increase in the number of operating automobiles over the last 50 years has greatly accelerated deposition of lead in the marine environment.

The concentration of lead fallout from the atmosphere depends on both the physico-chemical form of the lead and on meteorological conditions. Chow *et al.* (1970) have reported however, that a major portion of airborne lead falls out near the source. This is reflected in the concentrations of lead measured in saline waters by Fishbein (1974). He found lead concentrations of 0.015 µg l^{-1} in open-ocean water and up to 0.2 µg l^{-1} in nearshore waters. Coastal and estuarine organisms are thus most threatened by lead pollution of marine waters. Since little is known about the impact of low lead concentrations on oysters, we initiated studies which exposed *Crassostrea virginica* to various concentrations of lead under ambient conditions. Pringle *et al.* (1968) and Shuster and Pringle (1969) provide the only published data on the uptake of lead by the American oyster. Their studies were, however, conducted under controlled laboratory conditions and with concentrations of lead (25 to 200 µg l^{-1}) that far exceeded those that would normally be expected in the environment. Determination of meaningful quality criteria for lead will depend on research that employs more relevant environmental concentrations. Our experiments with oysters were conducted under conditions similar to natural ones with respect to physico-chemical conditions (i.e., temperature and salinity) and lead concentrations.

Our study focused on the kinetics of bioaccumulation and depuration of lead

0025-3162/79/0052/0189/$01.60

by oysters over an extended period of time. In addition, we examined the effects on gamete and larval development during treatment with lead. The ultimate aim of our studies was to establish *Crassostrea virginica* as a biological indicator of lead in nearshore marine waters and to determine the impact that lead has on larval production and survival in this species.

Materials and Methods

Bioaccumulation

Test organisms were adult oysters, *Crassostrea virginica*, obtained from Long Island Sound, USA, in March, 1975. The mean height of the sample population was 9.8 cm (range 9.2 to 10.8 cm). The general health condition of the oysters was determined by histological examination at the time of harvest and at the start of lead addition to the experimental troughs. In addition, a sample of 50 oysters was taken to determine if a relationship existed between dry weight and lead concentration or content. All other oysters were acclimatized for 1 month in fiberglass troughs with a flow-through seawater system using raw Narragansett Bay water. The troughs measured 3.75 m long x 30 cm wide x 25 cm deep and were supplied with flowing unfiltered seawater (29 to 32‰ S) at the rate of 16 l min^{-1}. Each trough held 50 oysters on a false bottom consisting of polyethylene grids resting 2.5 cm off the trough bottom on PVC pipe. The oysters did not receive supplementary feeding during the holding or treatment periods since the incoming seawater contained ample food organisms. The ambient seawater temperature was recorded continuously during the experiment.

Eight troughs (4 per lead concentration) received a lead nitrate [$Pb(No_3)_2$] solution, and 4 troughs served as controls. A deionized water stock-solution was pumped into the troughs at a rate which, when mixed with incoming seawater (16 l min^{-1}) produced a calculated concentration of 1.0 or 3.3 µg Pb kg^{-1}.

An additional 4 troughs (200 oysters) were used in a depuration study. Two troughs received lead (3.3 µg kg^{-1}) for 20 weeks and 2 served as controls. After 20 weeks, addition of lead to the test troughs was terminated and untreated seawater was used for the following 12 week depuration period.

Seawater lead concentrations in the experimental troughs were determined weekly using methods described by Davey and Soper (1975). Concentrations ranged from 0.87 to 1.04 µg kg^{-1} and 3.21 to 3.46 µg kg^{-1} for the 1.0 and 3.3 µg kg^{-1} lead treatments, respectively. Seawater in the control troughs contained 0.12 to 0.16 µg Pb kg^{-1}. No mortalities were observed throughout the study.

Five oysters were removed from each treatment and the lead concentration determined prior to dosing with lead in May. During the accumulation period, 5 oysters were removed and analyzed weekly for 20 weeks. In the depuration experiment, the first sample was taken after 20 weeks treatment, and thereafter weekly samples were taken for 12 weeks.

Oysters to be analyzed by atomic absorption spectrophotometry were shucked, shell liquor removed, and the total soft parts were oven-dried at 100°C prior to wet-ashing in concentrated HNO_3. The procedure followed was that reported by Pesch *et al.* (1977).

Multiple regression analyses were performed to determine what influence lead concentration, seawater temperature, time and weight had on uptake and depuration of lead.

Larval Study

Gametes were obtained from experimental oysters by methods similar to those described by Loosanoff and Davis (1963). Approximately 10 oysters were removed once weekly from each of the 3 test treatments and placed in individual glass dishes (10 x 20 x 6 cm) containing artificial seawater (Zaroogian *et al.*, 1969) and immersed in a temperature-controlled water bath. The oysters were spawned by alternating the water temperature between 18° and 28°C. Manipulation of the water temperature continued until a male and female from each treatment had spawned.

After they had settled to the bottom of the spawning dishes, eggs were withdrawn by pipette and placed in approximately 12 l of seawater containing ambient, 1.0, or 3.3 µg Pb kg^{-1}. All possible cross-fertilizations between treatments were obtained by mixing 1 or 2 ml of the appropriate sperm suspension with the selected egg-seawater treatments.

The eggs were concentrated approximately 1 h after fertilization by allowing them to settle to the bottom of a Pyrex beaker. A subsample of the newly fertilized eggs (ca. 0.5 ml in volume) was withdrawn by pipette and placed in a plastic culture dish containing 10 ml of the appropriate treatment water. These culture dishes (3 replicates per experimental condition were incubated at 20°C for 48 h. One ml of 10% buffered forma-

lin was added to each culture dish following the 48 h incubation period. The larvae were then examined microscopically and the percentage of abnormal individuals was determined on the basis of shell shape and development (Woelke, 1961).

The remaining newly fertilized eggs were placed in plastic containers containing 200 ml of the appropriate treatment water and incubated at 20°C for 48 h. Following incubation, each container was well mixed and a subsample of the larvae withdrawn. The percentage of abnormal individuals was then determined as described above. The remaining larvae were collected on a 54 µm screen and resuspended in 200 ml of new treatment water. Larvae from each treatment were fed an equal amount of the food organism *Isochrysis galbana*. The water was changed and food added twice weekly. Percent mortality and average size of live larvae were determined twice weekly for 4 weeks.

Results

Bioaccumulation

After 20 weeks treatment, there was a significant linear relationship between Pb uptake by *Crassostrea virginica* and Pb concentration in the seawater over the range of concentrations used in this study (Fig. 1). In addition, the mean concentration of lead in adult oysters was significantly different between treatments. As shown in Fig. 2, as much as 4.35 ± 1.41 and 13.0 ± 2.20 µg Pb g^{-1} dry weight were accumulated by adult oysters from seawater containing 1.0 and 3.3 µg Pb kg^{-1}, respectively after 10 weeks treatment. However, as evident from Fig. 2, the mean concentrations of lead in the tissues after 20 weeks treatment were: control: 1.64 ± 0.98 µg Pb g^{-1} dry weight; 1.0 µg Pb kg^{-1}: 6.57 ± 0.86; 3.3 µg Pb kg^{-1}: 11.42 ± 2.88.

Regression analyses showed that slopes for both temperature and time of exposure increased with increase in concentration of Pb in the troughs. This was not unexpected, since total Pb uptake increased with increased exposure concentration and increasing ambient temperature. All equations were significant at P = 0.05. The multiple correlation coefficient (R^2), which is a measure of how much variability can be explained by the regressive model, also increased with lead concentration. When oysters were exposed to lead (3.3 µg kg^{-1}), temperature alone accounted for 39% of the total variability in the system, whereas time of exposure accounted for 30%. Because of the correlation between time of exposure and seawater temperature, it was not clear which of these was the more dominant factor affecting lead uptake.

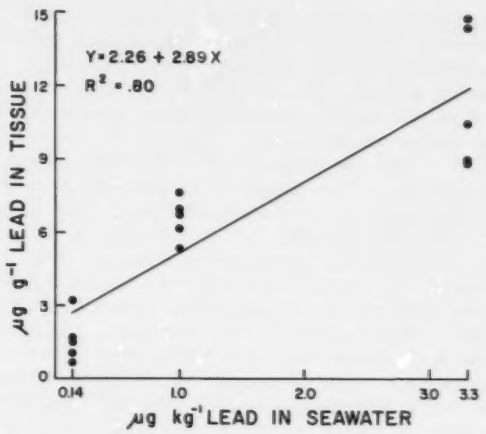

Fig. 1. *Crassostrea virginica*. Lead uptake after 20 weeks treatment, as a function of lead concentration in seawater

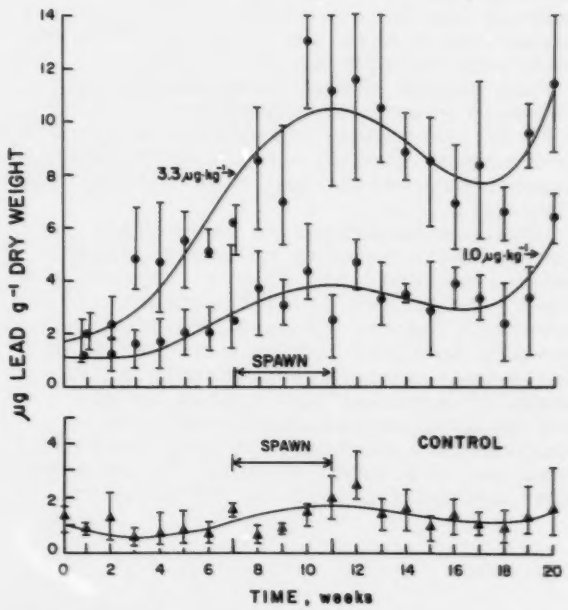

Fig. 2. *Crassostrea virginica*. Lead uptake from seawater containing ambient (control), 1.0 and 3.3 µg Pb kg^{-1}. Exposure period started in May 1975 and terminated in October 1975. Each symbol represents mean (circle) and minimum and maximum (bar around mean) concentrations for each sample of 5 oysters

Although significant linear relations between time and uptake existed, the R^2 values for these linear models were usually small (0.28 and 0.30 for the 1 and 3.3 µg kg^{-1} treatments, respectively), indicating that little of the variability can be explained by the linear regression. The significance of the linear regression indicated that the general trend of uptake as a function of time was increasing (positive slopes for all lines). For both treatment concentrations, there is a general increasing trend. In an effort to further illustrate the relation of time and uptake, polynomial regressions of uptake onto time were investigated. The R^2 values for the linear regression model of uptake means and time of exposure for the 1.0 and 3.3 µg kg^{-1} exposures were 0.48 and 0.45, respectively; whereas, the R^2 values for a fourth-degree polynomial were 0.75 and 0.82, respectively. Both of these regressions were obtained with mean uptake values. Fig. 2, which is the plot of the fourth-order fits, illustrates the uptake patterns obtained for both lead treatments and controls.

Dry weight had no significant relationship with lead concentration (µg g^{-1}) in the tissues; however, a significant direct linear relationship existed between dry weight and lead content (µg) (Fig. 3).

Oysters continued to accumulate lead during their natural spawning period, which occurred from July 21 to August 8 (Weeks 7 through 11 of treatment) in our treatment tanks, although residues greatly declined during some weeks in the 4-week period. Immediately after spawning activity had ceased in mid-August and, continuing to the end of September, a decrease in body burden of lead was observed, despite the continuous addition of lead to the seawater.

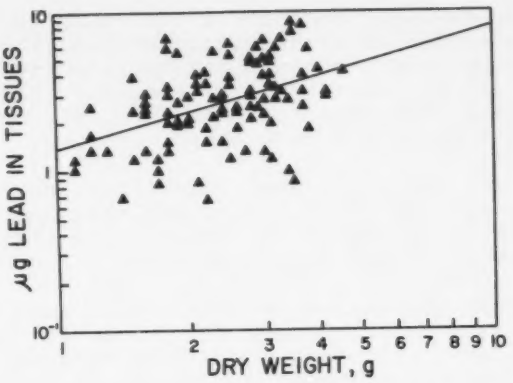

Fig. 3. *Crassostrea virginica*. Lead content (µg) as a function of dry weight in sample of 50 oysters taken at same time that oysters were collected for uptake study

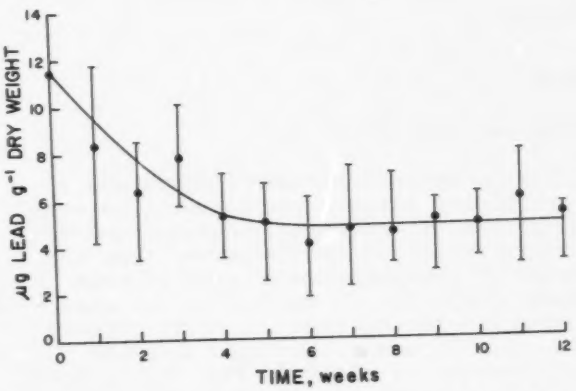

Fig. 4. *Crassostrea virginica*. Depuration of lead from soft tissues. Depuration started in October 1975 and terminated in January 1976

Depuration

Depuration of lead by adult oysters was curvilinear and appeared to proceed in an exponential decay function for the first 4 to 5 weeks. During this time approximately 54% of the lead body burden was depurated (Fig. 4). The biological half-life of lead in oyster soft tissue was calculated to be 5.5 weeks employing the method developed by Renfro (1973) and used by Cunningham and Tripp (1975) to calculate the half-life of mercury in oysters. The following equation was used:

$$T_{B/2} = \frac{\log^2}{b},$$

where $T_{B/2}$ is equal to the biological

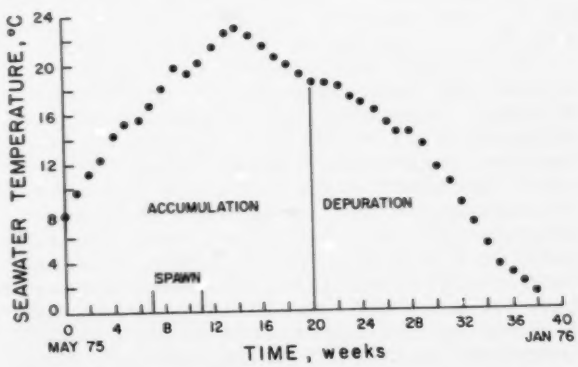

Fig. 5. Weekly mean seawater temperatures during lead treatment and depuration

half-life in weeks and b = the slope of the regression line having the equation $\log Y = 2.03 - 0.054X$. The lead depuration experiment exhibited significant linear regression lines of time and temperature of the seawater with lead concentration. There was an inverse relation of time with lead concentration, i.e., lead concentration decreased over duration of the experiment. The temperature correlation was positive, since seawater temperature was decreasing during the time of depuration (Fig. 5).

Larval Study

Oysters were induced to spawn for a 5 week period starting July 21. Larvae from each spawn developed to the straight-hinged stage within 48 h. The number of abnormals in all groups was never greater than 5%, which is well within the acceptable error of the test. No differences were observed among larval treatments or cross-fertilization combinations.

Length of the larvae from all treatments ranged between 105 and 110 µm after 4 weeks. Again, no differences in size were observed between any of the treatments.

Discussion

Although we found uptake of lead in adult *Crassostrea virginica* to be a curvilinear function, linear analyses showed that the general trend was an increase in body burden of lead. The significant loss of lead that occurred between 11 and 18 weeks influenced our findings that uptake was curvilinear despite the general trend of an increase in tissue lead concentration. Spawning or other metabolic fluctuations may have been the reason for this discrepancy.

The observed decline in body burdens of lead in oysters which occurred after the natural spawning period, could possibly have been an effect or result of spawning. The spawning process may have caused temporary loss of the oyster's capacity to retain or accumulate lead. Perhaps the oysters did not resume accumulation of lead until they had completed post-spawning metabolic adjustment, when synthesis had occurred. Cunningham and Tripp (1973) found mercury uptake to be curvilinear in *Crassostrea virginica*, perhaps due to the influence of spawning activities. However, in a later study, Cunningham and Tripp (1975) established that mercury uptake in *C. virginica* was curvilinear when spawning did not disrupt accumulation.

The decline in lead residues could be attributable to variations in weight, since higher concentrations of metals have been reported in smaller oysters than in larger individuals (Cunningham and Tripp, 1975; MacKay *et al.*, 1975). However, we found no significant relation between tissue concentration and dry weight.

In addition to temperature, other seasonal influences may have affected our results as well. Galtsoff (1964), for example, recognized patterns of natural decline in metal concentration in oysters which he attempted to relate to glycogen concentrations. Frazier (1975) reported that the quantity of metal lost from mid-August to mid-September comprised a significant fraction of the total body burden in oysters.

Bryan (1973) noticed seasonal variation in metal concentration in *Pecten maximus* and *Chlamys opercularis* and observed highest values in autumn and winter months. He observed that metal concentrations in tissues were generally highest when phytoplankton productivity was low, and suggested that although the heavy metal concentration in seawater influences accumulation of heavy metals by bivalves, phytoplankton productivity may be more important in bioaccumulation of metals.

If our uptake studies had been terminated by early August, it is quite possible that we would have observed only a linear uptake of lead by oysters. Schulz-Baldes (1974), for example, found that *Mytilus edulis* accumulated lead linearly; but her experiment lasted only 40 days and the temperature was maintained at 12°C during the study. We feel, based on our observations, that both pre- and post-spawning periods must be included in uptake studies to accurately demonstrate whether uptake is a continuous, consistent process, when attempting to establish water-quality criteria. If water-quality criteria were determined from uptake studies which incorrectly presumed uptake to be a continuous linear process, the values derived could, in fact, be inflated.

Phillips (1976) has described the requisites for a biological indicator of trace metals. Included are: a sedentary or sessile existence, abundance in the study region, longevity and ease of sampling, tolerance of brackish water, accumulation of pollutants with a high concentration factor to allow direct analysis, and a simple correlation existing between the metal content of the organisms and the average metal concentration in the surrounding water. He suggested that the last is the most im-

portant requirement and should be satisfied under the entire range of environmental conditions experienced by the organism to be used as an indicator. In addition to these requirements, we would also include that the organism should be relatively insensitive to the adverse effects of the metals at the concentrations to be measured.

We feel that *Crassostrea virginica* satisfies these requirements for lead. Also, if oysters or other shellfish are to be used as biological indicators of metal pollution, proper determinations of metal concentration could only be derived from samples taken continuously over an extended period of time, including a spawn. This is exemplified by the differences we observed in amounts of lead accumulated in tissues after 10 and 20 weeks at 3.3 µg Pb kg^{-1}. Lead concentrations were lower at the later sampling period due to the decrease in body burdens between Weeks 11 and 18 of testing. However, since linear regression analyses indicated a general tendency for oysters to increase body burdens of lead over time, we feel that concentrations would continue to increase to levels even higher than those we observed prior to spawning.

Pringle *et al.* (1968) reported that little lead was lost from *Crassostrea virginica* when tissue residues were greatest. They established that after 21 days at constant temperature (20°C ± 1 C°), *C. virginica* with the smallest body burdens depurated 79% of the lead while those with the highest body burdens lost only 7%. In addition, they indicated that lead was released at a constant rate. A pattern which was observed by Cunningham and Tripp (1973) in a study of mercury uptake by *C. virginica* was consistent with our findings for lead: They observed during a decline in ambient seawater temperature that 21% of the accumulated mercury was depurated within the first 18 days following exposure and that no loss occurred after that post-exposure time interval. In a later study, Cunningham and Tripp (1975) established that in a declining temperature regime a 56% decrease in mercury was observed within 45 days; and after 80 days, 79% was eliminated by oysters previously exposed to 10 ppb mercury for 45 days. Oysters which had received 100 ppb mercury for 45 days lost 66% within 45 days and 92% after 80 days. When depuration was studied at a mean temperature of 25°C, no significant decline in mercury occurred in the first 14 days after a 45-day exposure to 10 ppb mercury. When oysters had received 100 ppb mercury for the same time, a 51% decline in mercury was observed after 14 days depuration.

Here, we have shown that *Crassostrea virginica* containing lead body burdens of 11.4 µg g^{-1} lost 54% of the lead after 4 weeks under conditions of natural temperature decline. In addition, lead did not appear to be released at a constant rate.

Based on the results of our study, we postulated that depuration of lead by oysters was not entirely time- and temperature-dependent. Rather, we felt that depuration occurred rapidly until a residual concentration of lead in tissues was attained, after which the lead loss occurred slowly, if at all. It is our contention that this residual concentration of lead in the tissues was determined by the amount of lead which was bound and not released easily, if at all, whereas the lead which was loosely bound or unbound (interstitial) would be depurated more easily and its loss could be governed by metabolic activity.

Chemical species of the metal is also a very important consideration in bioaccumulation studies, as was recognized by Schulz-Baldes (1974) when she indicated that the chemical form of lead in the medium influenced the rate of uptake by mussels. Cunningham and Tripp (1975) observed a four-fold increase in mercury accumulation by oysters when organic mercury was substituted for mercuric chloride. Coombs (1977) and George and Coombs (1977) reported that prior chelation of lead increased both the rate of accumulation and the final tissue concentrations in *Mytilus edulis*.

In this study, 85 to 90% of the lead added to the seawater in the troughs was detected in the particulate fraction. The remainder of the lead probably occurred in a complexed inorganic and a chelated form in the soluble fraction. Any ionic species would probably be adsorbed to the surfaces of the troughs (G. Hoffman, personal communication).

The adsorptive behavior of metals is strongly pH-dependent, and the metal-binding ability of a ligand decreases when the pH of the medium is lowered (Perrin and Agarwal, 1973). Thus, lead could be released from the particulates and a shift could occur in the equilibrium between the chemical species of lead. This change in equilibrium could favor increased ionic lead in the digestive tract (pH 5.5 to 6.0: Galtsoff, 1964). The amount of ionic lead made available in the digestive tract or other tissues could determine the concentration of lead that is bound firmly and released very slowly, if at all. It is also possible that complexed and chelated lead is loosely bound or unbound

(interstitial) and represents that fraction of lead which is lost from the tissues more easily.

Coombs (1977) suggested that metal route of entry in *Mytilus edulis* is by endocytosis in the gill and gut. Cunningham and Tripp (1975) in addition to Coombs, reported in *Crassostrea virginica* and *M. edulis*, respectively, that metal transport to other tissues for storage and excretion could be via circulating amoebocytes.

Calabrese *et al.* (1973) studied the effect of heavy metals on the survival of embryos of *Crassostrea virginica*, and reported a 48 h LC_{50} of 2.45 µg g^{-1} for lead. Their study was designed to determine the acute toxicity of metals to oyster embryos obtained from adults held in untreated seawater. Our study was designed to determine if embryos from treated parents were more sensitive to lead toxicity than embryos from untreated parents. In addition, in order to determine if male or female gametes were selectively affected by lead, all possible crosses with gametes from treated oysters and controls were made. It appeared that the concentrations of lead in the seawater used and the body burdens recorded in this study had no apparent affect on the reproductive process.

Conclusions

Crassostrea virginica is an excellent bioindicator of lead, since tissue concentrations increased when exposed to seawater containing 1.0 µg Pb kg^{-1}. Lead uptake is curvilinear; however, the general trend is an increase over time. Lead-uptake studies with oysters should include pre- and post-spawn periods in order to obtain a complete picture. Depuration of lead was curvilinear, did not appear to be temperature-dependent, nor was it complete. Under the conditions of this experiment, lead appeared to have no effect on oyster larval viability or development.

Acknowledgements. We acknowledge Mr. W. Galloway and Mr. J. Sewall for their skilled technical assistance, Mr. R. Hennekey for review of the manuscript, and Dr. E.W. Davey and Mr. A.E. Soper for the seawater analyses.

Literature Cited

Bryan, G.W.: The occurrence and seasonal variation of trace metals in the scallops *Pecten maximum* (L) and *Chlamys opercularis* (L). J. mar. biol. Ass. U.K. *53*, 145-166 (1973)

Calabrese, A., R.S. Collier, D.A. Nelson and J.R. MacInnes: The toxicity of heavy metals to embryos of the American oyster *Crassostrea virginica*. Mar. Biol. *18*, 162-166 (1973)

Chow, T.J., R.H. Motto and D.M. Chilko: Lead in the marine environment. Envir. Sci. Technol. *4*, 318-322 (1970)

Coombs, T.L.: Uptake and storage mechanisms of heavy metals in marine organisms. Proc. analyt. Div. chem. Soc. *14*, 219-222 (1977)

Cunningham, P.A. and M.R. Tripp: Accumulation and depuration of mercury in the American oyster *Crassostrea virginica*. Mar. Biol. *20*, 14-19 (1973)

--Factors affecting the accumulation and removal of mercury from tissues of the American oyster *Crassostrea virginica*. Mar. Biol. *31*, 311-319 (1975)

Davey, E.W. and A.E. Soper: Apparatus for the sampling and concentration of trace metals from seawater. *In:* Analytical methods in oceanography, pp 16-21. Ed. by T.R. Gibb. Washington, D.C.: American Chemical Society 1975

Fishbein, L.: Mutagens and potential mutagens in the biosphere. II. metals — mercury, lead, cadmium and tin. Sci. total Envir. *2*, 341-371 (1974)

Frazier, J.M.: The dynamics of metals in the American oyster, *Crassostrea virginica*. 1. Seasonal effects. Chesapeake Sci. *16*, 162-171 (1975)

Galtsoff, P.: The American oyster, *Crassostrea virginica* (Gmelin). Fishery Bull. Fish. Wildl. Serv. U.S. *64*, 1-480 (1964)

George, S.G. and T.L. Coombs: The effects of chelating agents on the uptake and accumulation of cadmium by *Mytilus edulis*. Mar. Biol. *39*, 261-268 (1977)

Loosanoff, V.L. and H.C. Davis: Shellfish hatcheries and their future. Comml Fish. Rev. *25*, 1-11 (1963)

MacKay, N.J., R.J. Williams, J.L. Kacprzac, M.M. Kazacos, A.J. Collins and E.N. Aruty: Heavy metals in cultivated oysters (*Crassostrea commercialis = Saccostrea cucullata*) from the estuaries of New South Wales. Aust. J. mar. Freshwat. Res. *26*, 31-46 (1975)

Perrin, D.D. and R.P. Agarwal: Multimetal-multiligand equilibria: a model for biological systems. *In:* Metal ions in biological systems. Vol. 2. Mixed-ligand complexes, pp 168-202. Ed. by H. Sigel. New York: Marcel Dekker Inc. 1973

Pesch, G., B. Reynolds and P. Rogerson: Trace metals in scallops from within and around two ocean disposal sites. Mar. Pollut. Bull. *8*(10), 224-228 (1977)

Phillips, D.J.H.: The common mussel *Mytilus edulis* as an indicator of pollution by zinc, cadmium, lead and copper. I. Effects of environmental variables on uptake of metals. Mar. Biol. *38*, 59-69 (1976)

Pringle, B.H., D.E. Hissong, E.L. Katz and S.T. Mulawka: Trace metal accumulation by estuarine molluscs. J. sanit. Engng Div. Am. Soc. civ. Engrs *94*, 455-475 (1968)

Renfro, W.C.: Transfer of ^{65}Zn from sediments by marine polychaete worms. Mar. Biol. *21*, 305-316 (1973)

Schulz-Baldes, M.: Lead uptake from seawater and food, and lead loss in the common mussel *Mytilus edulis*. Mar. Biol. *25*, 177-193 (1974)

Shuster, C.N. and B.H. Pringle: Trace metal accumulation by the American eastern oyster, *Crassostrea virginica*. Proc. natn. Shellfish. Ass. *59*, 93-103 (1969)

Woelke, C.E.: Bioassay — the bivalve larvae tool. Proc. Pacif. NW Symp. Wat. Pollut. Res., U.S. publ. Hlth Serv., Portland, Ore. *10*, 113-123 (1961)

Zaroogian, G.E., G. Pesch and G. Morrison: Formulation of an artificial seawater medium media suitable for oyster larvae development. Am. Zool. *9*, p. 1144 (1969)

Dr. G.E. Zaroogian
Environmental Research Laboratory
U.S. Environmental Protection Agency
South Ferry Road
Narragansett, Rhode Island 02882
USA

Date of final manuscript acceptance: February 16, 1979. Communicated by M.R. Tripp, Newark